

ImmunoTools *special* Award 2014



Teresa Ciudad, PhD-student

Supervisor: Prof. Dolores Jaraquemada

Laboratorio de Inmunología Celular, Instituto de Biotecnología y Biomedicina, Universidad Autónoma de Barcelona, Campus Bellaterra, 08193 Bellaterra, Barcelona, Spain

IMPACT OF TISSUE-SPECIFIC PROCESSING OF THYROGLOBULIN (Tg) ON HLA-DR3-ASSOCIATED Tg PEPTIDE REPERTOIRES.

Tissue-specific conditions together with the specific properties of the disease-associated HLA alleles, may generate an in situ peptide repertoire with enough differences from the repertoire in thymus to activate a good number of otherwise low-affinity autoreactive T cells. This hypothesis is supported by type-B T cells that recognize peptide-MHC complexes that are different from those generated when peptide is derived from intracellular processing of native protein.

The study of peptides repertoires associated to HLA-DR in thymus and autoimmune tissue (i.e Graves' disease, reumatoid arthritis, multiple sclerosis) demonstrated differences in processing and antigen presentation. When theoretical affinity for HLA-DR is compared, it is shown that 75% of peptides from thymus are high binders whereas in thyroid the majority of peptides are intermediate and low binders. These data support the relevance of affinity in tolerance: in thymus a wide range of peptides must be presented, so competition favors the highest affinity peptides. However, in periphery, peptides may be generated for tissue-specific processes but not necessarily to be presented by MHC II molecules, so affinity would not be so important. The aim of this work is to prove that differences in antigen processing between immune tissue and peripheral target tissue in autoimmunity generate differences in peptide repertoire, specially in peptides involved in immune response. Our hypothesis is that protease expression and their role in intra or extracellular antigen processing are relevant in autoimmunity.

For MHC-II molecules, normal turnover of tissue-specific proteins, extracellular processing and different tissue proteases may provide a source of peptides in the target tissue that will not be found in thymus. The cleavage of thyroglobulin (Tg) in the thyroid is a good example. Tg is produced by thyroid follicular cells (TFC) and secreted into the colloid. Solubilization and pre-cleavage of Tg are necessary prior to endocytosis by TFC to generate T3 and T4 hormones. Cat B, L, S, K present both in colloid and in the TFC's endocytic vesicles cleave Tg at different pH (neutral and acid pH, respectively), giving a different pattern of cleavage in each condition. We have

identified Tg peptides that are associated in vivo to HLA-DR molecules in human autoimmune Graves' disease and at least one of these peptides (Tg2098) has been demonstrated to be immunodominant.

Using thyroid autoimmune disease as a model, we have simulated processing conditions of thyroglobulin in thyroid and thymus using different sets of cathepsins analyzing the HLA-DR3-associated thyroglobulin peptides. We have used a cell-free system that mimic the lysosomal compartments where antigens are degraded. Cathepsin B, H and S were included as the essential proteases needed for antigen processing by dendritic cells (DCs) at acid pH. In addition, we included thyroglobulin partially degraded in colloid at neutral pH, prior processing by DCs. As expected, quantitative and qualitative differences in peptide repertoires were observed. However, it is specifically relevant that the immunodominant peptide (Tg2098) was only isolated when thyroglobulin is pre-processed in colloid-like conditions, suggesting that tissue-specific events may generate peptides that are not presented in thymus for central tolerance (MT Ciudad et al, in preparation).

Results from the cell-free system will be compared with the peptide repertoire generated by dendritic cells (DCs). Since infiltrating DCs are not abundant in autoimmune tissues, we will set up the methodology to purify peptides associated to MHC-II molecules from monocyte-derived DCs (with IL-4 and GM-CSF) pulsed with a given antigen. In our studies, HLA-DR3⁺ monocyte-derived DCs will be pulsed with thyroglobulin (whole protein or predigested) or thyroid extracts (from the same thyroid sample where Tg2098 was identified). Moreover, peptides will be test in activation assays. CD4⁺ T cells will be isolated from thyroid infiltrating T cells from HLA-DR3⁺ patient's thyroids. Cytokine expression (IFN γ , TNF α and IL-4) and cell proliferation will be measured.

ImmunoTools special AWARD for Teresa Ciudad includes 25 reagents

FITC - conjugated anti-human HLA-DR,

PE - conjugated anti-human CD4, CD8, CD14,

PerCP - conjugated anti-human CD3,

APC - conjugated anti-human CD3, CD11c, CD19,

CD4 **FITC** / CD3 **PE** / CD8 **PerCP**

CD3 **FITC** / CD4 **PE** / CD45 **PerCP**

human ELISA-set for 96 wells, human IFN-gamma, human IL-4 and human TNF- α (each 3 reagents),

recombinant human cytokines: rh GM-CSF, rh IL-4, rh IL-2, rh TNF α , rh IL-13, rh IFNgamma

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